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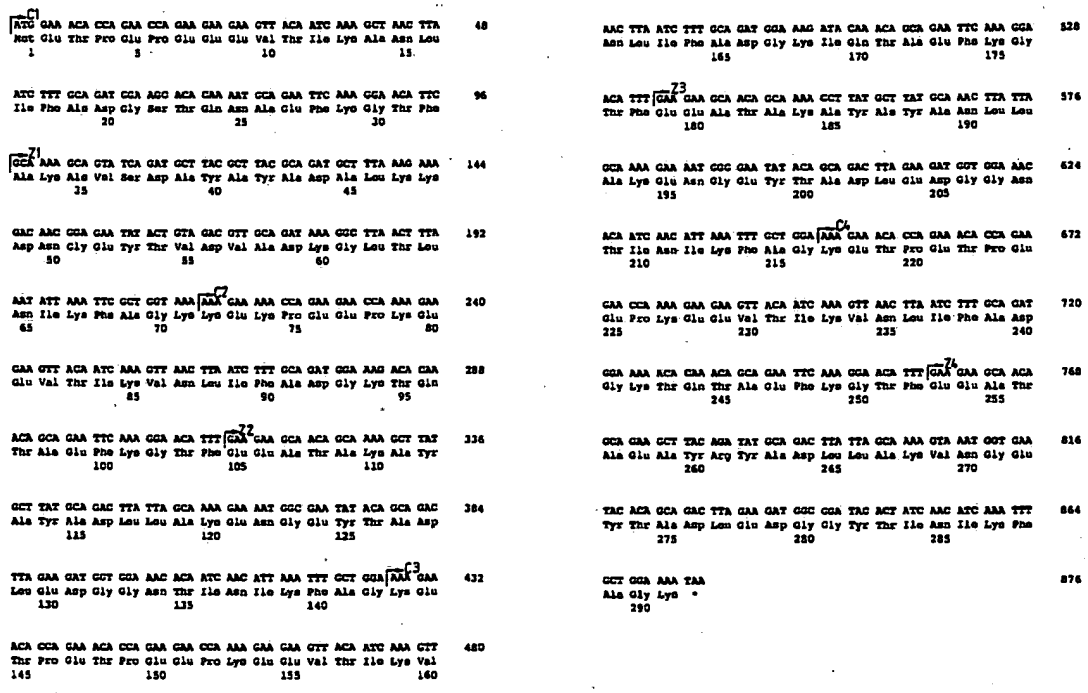

(57) Abstract

Synthetic molecules having one or more binding regions with binding affinity for Kappa light chains of immunoglobulins, processes for their production and recombinant DNA coding therefor.

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Immunoglobulin binding proteins derived from L protein and their uses

This invention relates to novel immunoglobulin binding proteins, processes for their production and recombinant DNA molecules coding therefor.

More specifically the present invention relates to synthetic proteins containing repeated sequences derived from selected binding regions of Protein L and to recombinant DNA molecules coding therefor.

A multitude of Gram-positive bacteria species have been isolated that express surface proteins with affinities for mammalian immunoglobulins through interaction with their heavy chains. The best known of these immunoglobulin binding proteins are type 1 Staphylococcus Protein A and type 2 Streptococcus Protein G which have been shown to interact principally through the C2-C3 interface on the Fc region of human immunoglobulins. In addition, both have also been shown to interact weakly to the Fab region, but again through the immunoglobulin heavy chain.

Recently, a novel protein from *Peptococcus magnus*, Protein L, has been reported that was found to bind to human, rabbit, porcine, mouse and rat immunoglobulins uniquely through interaction with their light chains. In humans this interaction has been shown to occur exclusively to the kappa chains. Since both kappa and lambda light chains are shared between different classes, Protein L binds strongly to all human classes, in particular to the multi-subunited IgM, and similarly is expected to bind to all classes in species that show Protein L light chain binding.

Both peptococcus and peptostreptococcus have been reported to produce Protein L, which binds to the Kappa light chain of human immunoglobulins. It has been proposed that Protein L is a virulence factor; non-virulent peptococci and peptostreptococci appear to neither express Protein L nor have the structural gene for it (Kastern *et al* 1990).

Protein L is of particular interest since it has been reported to bind to the Kappa light chain which is present in all classes and sub classes of immunoglobulins. As such it should prove to be a useful diagnostic reagent for use in ELISA and RIA techniques.

EP-A-0 255 497 describes the purification and attempted characterisation of Protein L by standard protein purification techniques. Subsequently, the authors of EP-A-0 255 497 have published a number of scientific papers describing further investigations into the nature and structure of Protein L, but to date, attempts fully to characterize the protein have failed. Thus recently, in a paper entitled "Protein L a Bacterial Immunoglobulin-Binding Protein and Possible Virulence Determinant" by W. Kastern et al (Infection and Immunity, May 1990, pp. 1217-1222) there are described unsuccessful attempts to isolate the gene coding for Protein L by determining N-terminal amino acid sequences of tryptic fragments of Protein L and using the derived sequence information to construct probes for isolating the gene. Although Protein L is useful for its immunoglobulin binding properties it is desirable to identify whether particular regions of Protein L confer immunoglobulin binding so that these regions may be used as the basis for construction of synthetic and improved immunoglobulin binding molecules. Due to lack of sequence information, it has hitherto not been possible to identify the Protein L sequences associated with complex formation with immunoglobulin Kappa light chains.

Hitherto, the problem of isolating and characterising the gene for Protein L has defined solution thereby preventing significant improvement in production of Protein L and preventing development of synthetic molecules derived from Protein L.

This invention is based on a cDNA sequence comprising a cDNA insert coding for Protein L in its entirety which has now been isolated, thus enabling the above problems to be solved. This cDNA sequence, and the amino acid sequence corresponding to the longest open reading frame thereof, are depicted in Figure 1. The beginning of the signal sequence is marked as "SS" and the beginning of the mature protein is marked as "M". The longest open reading frame of the sequence depicted in Figure 1 extends from TTG (103) to AAA(3183) and the depicted DNA comprises a coding region extending from nucleotide 208 to nucleotide 3183 which codes for immature Protein L.

The specific binding properties of Protein L, including its ability to bind immunoglobulin Kappa light chains, are believed to be attributable to the presence of sequences which have a recognisably repeated character within the amino acid sequence of the molecule.

By the term "recognisably repeated character" as used herein is meant that the amino acid sequence comprises at least two sequences, each of from 20 to 45 amino acids in length (or from 40 to 90 amino acids in length in the case of the D repeats), which have an at least 75%, preferably at least 90% and most preferably at least 95% homology with one another.

The polypeptide sequence depicted in Figure 1 includes various sets of repeated sequences at least two of which are considered to be responsible for immunoglobulin Kappa light chain binding.

These sets of repeated sequences are labelled at their N-terminal ends as follows:

(1) A1, A2 and A3;
(2) B1 and B2;
(3) C1, C2, C3, and C4;
(4) Z1, Z2, Z3 and Z4;
(5) D1, D2, D3 and D4;

Each of the repeated sequences (1) - (4) has a length of between 25 and 45 amino acids. The ability to bind Kappa light chains is considered to be associated with one or more of the repeated sequences A, B, C and Z (sequences (1) - (4) above).

It is thus a feature of a first aspect of the invention to provide synthetic immunoglobulin binding molecules comprising a plurality of recognisably repeated binding domains selected from the sequences which are labelled at their N-terminal ends in Figure 1 as A1, A2 and A3; B1, and B2; C1, C2, C3, and C4; and Z1, Z2, Z3 and Z4. The synthetic immunoglobulin binding molecules preferably comprise from 2 to 15 of said domains. The selected domain or domains may be identical to the sequences which are labelled at their N-terminal ends in Figure 1 as A1, A2 and A3; B1, and B2; C1, C2, C3, and C4; Z1, Z2, Z3 and Z4, or they may vary from said sequences, provided that they have an at least 75%, preferably at least 90% and most preferably at least 95% homology therewith.

The sequences labelled at their N-terminal ends as D1, D2, D3 and D4 are believed to be resposible for albumin binding and the synthetic binding molecules provided according to the invention may include sequences selected from sequences D1, D2, D3 and D4 or related squences which vary from said sequences, provided that they have an at least 75%, preferably at least 90% and most preferably at least 95% homology therewith.

In an embodiment of the invention hereinafter described a synthetic immunoglobulin binding molecule is provided in which domains C1 and Z1, and/or C2 and Z2 and/or C3 and Z3 and/or C4 and Z4 are present as binding region or regions. Region C1Z1 begins at the first amino acid of C1 and ends at the last amino acid of Z1, etc.

According to a further embodiment of the invention a synthetic immunoglobulin binding molecule comprises one or more immunoglobulin binding regions selected separately from:

(1) region C1Z1 of protein L,

(2) region C2Z2 of protein L,

(3) region C3Z3 of protein L,

(4) region C4Z4 of protein L, and

(5) a polypeptide sequence having at least 75% homology with one of the regions of (1), (2), (3) or (4) and substantially retaining the immunoglobulin binding activity of that region.

It is preferred that the synthetic molecule is substantially free of one or both of (1) protein L albumin binding activity and (2) protein L cell wall binding activity.

The sequence data shown in the figures indicate that regions C1Z1, C2Z2, C3Z3 and C4Z4 of protein L shown are respectively 71, 71, 74 and 75 amino acid residues in length. References in the invention to these regions are intended to encompass variants of these precise sequences. One such variant retains substantially the immunoglobulin binding activity of the precise sequence and has up to ten preferably up to 5 and very preferably no more than 2 amino acids substituted, added or deleted.

Another variant exhibits a degree of homology with one of the C1Z1, C2Z2, C3Z3 and C4Z4 sequences of 75% or more, preferably 90% or more while retaining substantially the immunoglobulin binding activity of the precise sequence.

The binding regions of the synthetic molecule are ligated directly to one another in one embodiment of the invention. In another embodiment binding regions are separated from each other by linker polypeptides, the nature of each linker being such as not to interfere with the binding activity of the binding domain. Linker polypeptides if present are preferably of up to 10 amino acids in length and most preferably up to 5 amino acids in length.

Although the invention includes synthetic molecules having a large number of binding regions it is convenient for the synthetic molecule to have from 1 to 4 such regions.

In a preferred embodiment of the invention the synthetic molecule has four such regions. The selection of a particular C_n Z_n or C_n Z_n-derived variant sequence for each of the four regions is optional. Thus the synthetic molecules of the invention cover a large number of possible combinations of C_n Z_n and C_n Z_n - derived variant sequences.

In a particular embodiment of the invention a synthetic molecule has four binding regions one each selected from C1Z1 or a variant thereof, C2Z2 or a variant thereof, C3Z3 or a variant thereof and C4Z4 or a variant thereof. An example of such an embodiment is shown in Fig 2 which binds to immunoglobulin as native Protein L but does not bind to albumin or cell wall as native protein L.

The synthetic molecules of the invention can conveniently be used to form products for use in protein analysis, purification procedures and other biochemical processes according to methods well known in the art.

The synthetic immunoglobulin binding molecules can, for example, be ligated to a "reporter" molecule, such as an enzyme so as to be suitable for enzyme linked immunoabsorbent assay (ELISA). In another example to "reporter" molecule is suitable for use in a chemiluminescent assay.

The synthetic molecules of the invention can additionally be ligated to a molecule suitable for attachment to a solid support, such as a cysteine residue for attachment to a further cysteine residue on a solid matrix, or histidine for attachment to zinc on a support, or a mussel derived adhesive protein for attachment to a wide variety of surfaces including glass.

Thus the invention provides novel synthetic immunoglobulin binding molecules that are useful in a wide range of biochemical applications. The synthetic molecules are of particular advantage if they are free from regions D1, D2, D3 and D4 and as a result they do not exhibit the albumin binding and cell wall binding of native protein L. The synthetic molecules of the invention can conveniently be used to form products for use in protein analysis, purification procedures and other biochemical processes according to methods well known in the art.

According to a second aspect of the invention there is provided a recombinant DNA molecule containing an insert coding for a synthetic molecule according to any embodiment of the first aspect of the invention.

A nucleotide sequence of an embodiment of the second aspect of the invention is shown in Fig. 2.

It is straightforward for a man skilled in the art, once in possession of the DNA sequence coding for a desired polypeptide, to construct a vector capable of transforming a host cell so as to express that polypeptide.

Thus, according to a third aspect of the invention there is provided a process for producing a synthetic molecule of the first aspect of the invention comprising the steps of

(a) transforming a host cell with an expression vector capable of transforming the host cell so as to express the synthetic molecule,
(b) culturing the transformed host cell, and
(c) isolating the synthetic molecule.

One such expression vector is plasmid pPPL2 described below and which has been deposited at NCIMB, Aberdeen, Scotland, UK under accession No. 40534 on 22 December 1992.

There now follows a description of exemplary embodiments of the invention in which:-

Fig.1 shows the nucleotide sequence of the gene coding for Protein L together with the amino acids coded for;

Fig. 2 shows the nucleotide sequence and the amino acid sequence coded thereby of an embodiment of the invention; and

Fig. 3 shows a schematic representation of two different Protein L isolates and deletion clones constructed to determine the function of the separate binding domains.

Fig. 3 shows 1.(a) Domain structure as determined by Kastern *et al.*, Infect. Immunol., 58, 1992, and 1.(b) domain structure as determined by Murphy *et al.*, Eur J. Biochem, 168, 1992. Shaded areas between the two figures represent areas of strong homology. To determine the domains responsible for the immunoglobulin-kappa binding reported for both molecules, and the albumin-binding reported for 1.(b), the deletion clones (constructed from the gene expressing 1.(b)) are shown in 2.(a,b,c).

Example 1

Materials

X-Omat S X-ray film was from Kodak. DNA ligase, restriction endonucleases and other DNA-modifying enzymes were from Boehringer. Agarose, acrylamide, bis-acrylamide and phenol were from Bethesda Research Laboratories. Chromatography media was from Pharmacia LKB (Uppsala, Sweden). All immunoglobulins and serum albumin were from Sigma. All other reagents were from Sigma of BDH. Nunc 96 well microtitre plates were purchased from Gibco BRL Ltd.

Media and Cutlure conditions

E.coli TG1 was cultured in 2xYT both (2% (w/v) tryptone/1% (w/v) yeast extract/1% (w/v) NaCl) overnight at 37°C. Media were solidified with 2% (w/v) Bacto-agar (Difco). Ampicillin (50µg/ml) were used where necessary for the selection and growth of transformants. Functional β-galactosidase was detected by addition of chlorindolyl-β-D-galactoside to a final concentration of 600 µg/ml and, where necessary, isopropyl-β-D-thiogalactopyranoside to a final concentration of 200 µg/ml.

Isolation of DNA

Plasmid DNA was purified from *E.coli* by Brij lysis (Clewell and Helsinki, PNAS, USA, 1969) and CsCl/ ethidium bromide density-gradient centrifugation (Radloff *et al.*, PNAS, USA 1967).

Genetic Manipulation Procedures

DNA-modifying enzymes were used in the buffer and under the conditions recommended by the supplier (Boehringer). Transformation of *E.coli* was essentially as described previously (Cohen *et al.*, PNAS, USA 1972). Electrophoresis of DNA fragments was performed on vertical 1% (w/v)-agarose slab gels in Tris-acetate buffer (40 mM-Tris/ /20mM-sodium acetate/ 2mM-EDTA, adjusted to pH 7.9 with acetic acid). DNA fragment sizes were estimated by comparison with fragments of lambda phage DNA previously digesed with the restriction endonuclease *Hind* III. DNA fragments were purified by electro-elution essentially as described previously (McDonnell *et al.*, J. Mol. Biol., 100, 1977).

Constriction of deletion clones

A schematic representation of the deletion clones constructed are shown in Fig. 3.

pPPL1 was constructed by amplifying the DNA fragment indicated in Fig. 3 (2a) isolating the A, B, C and Z repeats. To facilitate expression, an *Nde*1 site (CAT ATG) was incorporated into the sense primer (5'-TTA AAT CAT ATG TCA GAA ACA-3') and to prevent read through, a stop condon was incorporated into the anti-sense primer (5'-CC TGG TTG TTA TTT TCC AGC AAA T-3'). This fragment was cloned into the TA cloning vector (Amersham), and subsequently excised on a *Nde*1-partial *Hind* III (cleaving at the *Hind* III site present in the TA cloning vectors polylinker) fragment, and re-cloned inframe into the *Nde*1-*Hind* III cleaved expression vector pMTL1013 (Brehm *et al.*, Appl. Microbiol. Bitechnol., 36, 1991).

pPPL2, expressing only the C and Z repeats, was derived from pPPL1 by excision of the gene fragment shown in Fig. 3 (2b) through an *Eco* RV-*Spe*1 (site carried over from the TA cloning vector polylinker) digest, and re-cloned inframe into *Sma*1-*Xba*1 cleaved pMTL1013.

pPPL3 (Fig. 3 (2c)), expressing the D and E repeats, was obtained through a *Pst*1 (present upstream of the PPL open reading frame)-partial *Hind* III digest and cloned inframe into *Hind* III-*Pst*1 cleaved pMTL23 (Chambers *et al.*, Gene, 68, 1988).

PCR

PCR was achieved by synthesising oligonucleotides (synthesised by solid phase synthesis using an Appied Biosystems Model 380A DNA synthesiser employing phosphoamidites) either side of the target site on the PPL gene and DNA fragments generated by the polymerase chain reaction using the method and reagents supplied in the PCR-Perking Elmer Cetus GeneAmp^{TM} kit.

Sonication of cells

A cell suspension was transferred to a MSE sonication tube and subjected to ultra sonication (3x30 sec bursts at 18MHz with 30 sec intervals, at 4°C using an MSE Soniprep 150 Sonicator).

Affinity Chromatography on IgG-Sepharose 4B

The sonication procedure was used to disrupt bacterial cells for small scale purification of immunoglobulin-binding proteins by affintity chromatography on IgG-sepharose FF. Cultures of 300ml were grown overnight then centriguted (15000g for 10 min at 4°C) and resuspended in 3ml of 100 mM Tris-HCl, pH 7.5, 250 mM NaCl. The suspension was sonicated, centrifuged (30000g 10 min at 4°C) and the supernatant fluid passed through a 1ml column (1.6cm x 0.90cm i.d.) of IgG-sepharose FF equilibrated and washed with 5ml of 100 mM Tris-HCl, pH 7.5, 250mM NaCl. The protein was eluted with 100mM gylcine-HCl, pH 2.0, and the pH raised to 7.5 using 1M Tris, pH 8.0.

PAGE

Samples were solubilised under reducing condition and electrophoresis on SDS-polyacrylamide slab gels. Acrylamide (12.5% w/v) slab gels were run in an LKB vertical electrophoresis unit using the method of Laemmli (Laemmli, Nature, 227, 1970). Proteins were stained with Commassie Brilliant Blue R-250, and protein bands were scanned with a Chromoscan-3 laser optical densitometre (Joyce-Loebl, Gateshead, Tyne and Wear, U.K.), to estimate the apparent M_r.

Elisa Detecton assay

Immunoglobulin-binding proteins were detected using an Elisa procedure modified from that previously described (Warenes *et al.*, J. Immunol. Methods., 93, 1987).

Detection of immunoglobulin-binding

An aliquot of mouse IgG (100 µl) at 2.5µg/ml in 50mM sodium carbonate/bicarbonate buffer, pH9.6 was added to each well of a Maxisorp plate and the plate left overnight at 4°C. Following three washes with PBST-Phosphate buffered saline containing 0.05% (v/v) Tween 20, a 100µl aliquot of the suspension of recombinant bacteria was transferred to the

Maxisorp plate from overnight cultures. The immunoassay plate was then left at room temperature for an hour. After washing with PBST, 100µl of human IgG at 1µg/ml in PBST was added to each well and the plate left at room temperature for another hour. After a further wash, 100µl of goat anti-human IgG (Fc specific) horseradish peroxidase conjugate (diluted 1:2000 in PBST was added to each well and the plate left at room temperature for a further hour. After further washing, 100µl of reagent (60µg/ml 3,3', 5,5'-Tetramethylbenzine dihydrochloride, 0.003% (v/v) hydrogen peroxide in 0.1M sodium acetate buffer, pH6.0) was added to each well and the reaction allowed to proceed for 10min at room temperature. After this the reaction was stoped by the addition of 50µl 11% (v/v) sulphuric acid to each well. The absorbance of the wells were then read at 450nm against a reagent blank to measure the levels of immunoglobulin-binding proteins.

Detection of albumin-binding

To detect albumin-binding, the above procedure was followed except different affinity reagents were used in each step of the sandwich. The first step bound the protein sample under investigation, which has been prepared by recovering the cell supernatant following sonication, to the Maxisorp plate. Albumin-binding was then detected by incubating the plate with human serum albumin (HSA, 1µg/ml) followed by goat-anti HSA IgG-horseradish peroxidase conjugated (1:2000 dilution), and then developed as above.

The following results were obtained.

pPPL1 and pPPL2 (Fig. 2 (2a,b)) were shown by Elisa to bind to IgG, lacking any albumin binding. pPPL3 (Fig 2 (2c)) in contrast bound HSA, but not IgG. This shows that Kappa binding was through the C and Z repeats and that the albumin-binding was at a separate site located in the D- or E-repeats.

A purified solution of a synthetic immunoglobulin building molecule according to the invention can be obtained using the following method.

Host cells transformed with pPPL2 are grown, eg in a 400l to 4000l fermenter. The cell culture is then removed from the fermenter and spun down to obtain a cell paste, the supernatant culture medium being discarded.

The cell paste is washed in potassium phosphate buffer (pH 6.5) and lysosyme is added to lyse the cells over a suitable period of for example 30 to 60 minutes.

The lysed cells are next heated to 70°C for 15 minutes and then centrifuged at 13000 rpm for 2 hours, leaving a supernatant of soluble, crude protein which is removed from the centrifuged pellet and can be stored at -20°C.

To obtain a sample of the synthetic molecule the crude protein, either thawed from store or direct from centrifuging is eluted through a Q-Sepharose column previously equilibrated with potassium phosphate buffer (pH 6.5). Before being added to the column the crude protein solution is diluted so as to be at the same ionic strength as the buffer.

The column is washed with buffer until no more protein is washed off, then washed with 50mM NaCl solution to remove proteins binding weakly to the column. The strength of NaCl solution used to elute the column is then increased in steps and the protein fractions obtained kept separate.

The synthetic protein molecule of the invention is obtained from elution with NaCl between 270-290 mM.

The synthetic protein molecules of the invention, exemplified by that obtained as described above, find advantageous use in bio-assays and other biochemical applications due to their ability to bind to Kappa light chains of immunoglobulins. They are of use for example in ELISA, RIA, diagnosis, antibody purification.

Fig. 1 of GB 9209804.5 from which priority is claimed is reproduced as Fig. 1 of this application but with different nomenclature as set out below:

GB 9209804.5	This Application
A1, A2, A3	A1, A2, A3
B1, B2	B1, B2
C1, C2, C3, C4	C1, C2, C3, C4
D1, D2, D3, D4	Z1, Z2, Z3, Z4
E1, F1	*D1
E2, F2	*D2
E3, F3	*D3
E4, F4	*D4

This application uses the same nomenclature as the second priority application, GB 9226928.1.

*The sequence now marked as D1 consists of the sequence originally marked E1 together with the sequence orginally marked F1, etc.

CLAIMS

1. A synthetic immunoglobulin binding molecule comprising a plurality of recognisably repeated binding domains selected from the sequences which are labelled at their N-terminal ends in Figure 1 as A1, A2 and A3; B1, and B2; C1, C2, C3, and C4; and Z1, Z2, Z3 and Z4.

2. A synthetic molecule according to Claim 1 comprising from 2 to 15 of said domains.

3. A synthetic immunoglobulin binding molecule according to Claim 1 or Claim 2 wherein the selected domain or domains are identical to the sequences which are labelled at their N-terminal ends in Figure 1 as A1, A2 and A3; B1, and B2; C1, C2, C3, and C4; and Z1, Z2, Z3 and Z4, or vary from said sequences, provided that they have an at least 75%, preferably at least 90% and most preferably at least 95% homology therewith.

4. A synthetic molecule according to any of Claims 1 to 3 wherein said domains are from 20 to 45 amino acids in length.

5. A synthetic molecule according to any of Claims 1 to 4 additionally including domains selected from sequences D1, D2, D3 and D4 or related squences which vary from said sequences, provided that they have an at least 75%, preferably at least 90% and most preferably at least 95% homology therewith.

6. A synthetic molecule according to any of Claims 1 to 5 wherein the binding domains are selected from sequences C1, C2, C3, C4, Z1, Z2, Z3 and Z4.

7. A synthetic molecule according to Claim 6 comprising a binding region consisting of C1 linked at its C-terminus to Z1.

8. A synthetic molecule according to Claim 6 or Claim 7 comprising a binding region consisting of C2 linked at its C-terminus to Z2.

9. A synthetic molecule according to any of Claims 6, 7 or 8 comprising a binding region consisting of C3 linked at its C-terminus to Z3.

10. A synthetic molecule according to any of Claims 6 to 9 comprising a binding region consisting of C4 linked at its C-terminus to Z4.

11. A synthetic molecule according to any preceding claim in which all of domains D1, D2, D3 and D4 are absent.

12. A synthetic molecule according to any preceding claim comprising the amino acid sequence of Fig. 2.

13. A nucleotide sequence coding for a synthetic molecule according to any preceding claim.

14. A recombinant DNA molecule comprising a sequence according to Claim 13.

15. A method of producing a synthetic immunoglobulin binding molecule comprising the steps of

(1) forming an expression vector capable of transforming a host cell so as to express a polypeptide coded for by a DNA coding sequence according to Claim 14
(2) transforming a host cell with the vector
(3) culturing the host cell, and
(4) isolating substantially pure product from the host cell.

16. A method according to Claim 15 in which the vector is pPPL2.

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TTTGGACAGT GGACGAAACA AGAACACTGA TTTAATAAAT TGGTGAAATT CGATTGTTGA   60

AATACCTTTT TGGGTAGAAA TAACTAAGGA ATGGCAATAT AA TTG CTT GGA AAC     114
                                                Leu Leu Gly Asn
                                                -59

GAA TTT GAT TTA AAT AGC ATT AAA TGC AAA AAA TTT AAA AGG AGG AGA     162
Glu Phe Asp Leu Asn Ser Ile Lys Cys Lys Lys Phe Lys Arg Arg Arg
-55                 -50                 -45                 -40

CAA ATT CCA CCC TTT ATA AAG GGA AGT TTC CAT TGT CAA AAT AAT ATG     210
Gln Ile Pro Pro Phe Ile Lys Gly Ser Phe His Cys Gln Asn Asn Met
                -35                 -30                 -25
                                                          SS

AAG ATT AAT AAG AAA TTA TTA ATG GCT GCA CTT GCA GGA GCA ATT GTA     258
Lys Ile Asn Lys Lys Leu Leu Met Ala Ala Leu Ala Gly Ala Ile Val
            -20                 -15                 -10

GTT GGT GGT GGA GCT AAC GCT TAC GCA GCT GAA GAA GAT AAC ACT GAT     306
Val Gly Gly Gly Ala Asn Ala Tyr Ala Ala Glu Glu Asp Asn Thr Asp
         -5                  1                   5
                  M

AAT AAC CTT TCA ATG GAT GAA ATT AGT GAT GCT TAT TTT GAT TAT CAC     354
Asn Asn Leu Ser Met Asp Glu Ile Ser Asp Ala Tyr Phe Asp Tyr His
 10                  15                  20                  25

GGA GAT GTT TCA GAT TCA GTA GAT CCT GTA GAA GAA GAA ATA GAC GAA     402
Gly Asp Val Ser Asp Ser Val Asp Pro Val Glu Glu Glu Ile Asp Glu
                30                  35                  40

GCA TTA GCA AAA GCA TTA GCA GAA GCT AAA GAA ACA GCA AAA AAA CAT     450
Ala Leu Ala Lys Ala Leu Ala Glu Ala Lys Glu Thr Ala Lys Lys His
            45                  50                  55
                                                      A1

ATA GAT TCT TTA AAT CAT TTG TCA GAA ACA GCA AAA AAA TTA GCT AAG     498
Ile Asp Ser Leu Asn His Leu Ser Glu Thr Ala Lys Lys Leu Ala Lys
         60                  65                  70

AAT GAT ATA GAT TCA GCT ACT ACT ATT AAT GCA ATC AAT GAC ATC GTA     546
Asn Asp Ile Asp Ser Ala Thr Thr Ile Asn Ala Ile Asn Asp Ile Val
     75                  80                  85
                                                  B1

GCA AGA GCA GAT GTA ATG GAA AGA AAA ACA GCT GAA AAA GAA GAA GCA     594
Ala Arg Ala Asp Val Met Glu Arg Lys Thr Ala Glu Lys Glu Glu Ala
 90                  95                  100                 105
                                                          A2

GAA AAA TTA GCA GCA GCA AAA GAA ACA GCA AAG AAA CAT ATA GAT GAA     642
Glu Lys Leu Ala Ala Ala Lys Glu Thr Ala Lys Lys His Ile Asp Glu
                110                 115                 120

TTA AAA CAC TTA GCA GAC AAA ACA AAA GAA TTA GCT AAG AGA GAT ATA     690
Leu Lys His Leu Ala Asp Lys Thr Lys Glu Leu Ala Lys Arg Asp Ile
            125                 130                 135
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FIG.1

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GAT TCA GCT ACT ACT ATT AAT GCA ATC AAT GAC ATC GTA GCA AGA GCA     738
Asp Ser Ala Thr Thr Ile Asn Ala Ile Asn Asp Ile Val Ala Arg Ala
        140                 145                 150

GAT GTA ATG GAA AGA AAA ACA GCT GAA AAA GAA GAA GCA GAA AAA TTA     786
Asp Val Met Glu Arg Lys Thr Ala Glu Lys Glu Glu Ala Glu Lys Leu
    155                 160                 165

GCA GCA GCA AAA GAA ACA GCA AAG AAA CAT ATA GAT GAA TTA AAA CAC     834
Ala Ala Ala Lys Glu Thr Ala Lys Lys His Ile Asp Glu Leu Lys His
170                 175                 180                 185

TTA GCA GAC AAA ACA AAA GAA TTA GCT AAG AGA GAT ATA GAT TCA GCT     882
Leu Ala Asp Lys Thr Lys Glu Leu Ala Lys Arg Asp Ile Asp Ser Ala
                190                 195                 200

ACT ACT ATT GAT GCA ATC AAT GAT ATC GTA GCT AGA GCA GAT GTA ATG     930
Thr Thr Ile Asp Ala Ile Asn Asp Ile Val Ala Arg Ala Asp Val Met
            205                 210                 215

GAA AGA AAG TTA TCT GAA AAA GAA ACA CCA GAA CCA GAA GAA GAA GTT     978
Glu Arg Lys Leu Ser Glu Lys Glu Thr Pro Glu Pro Glu Glu Glu Val
        220                 225                 230

ACA ATC AAA GCT AAC TTA ATC TTT GCA GAT GGA AGC ACA CAA AAT GCA    1026
Thr Ile Lys Ala Asn Leu Ile Phe Ala Asp Gly Ser Thr Gln Asn Ala
    235                 240                 245

GAA TTC AAA GGA ACA TTC GCA AAA GCA GTA TCA GAT GCT TAC GCT TAC    1074
Glu Phe Lys Gly Thr Phe Ala Lys Ala Val Ser Asp Ala Tyr Ala Tyr
250                 255                 260                 265

GCA GAT GCT TTA AAG AAA GAC AAC GGA GAA TAT ACT GTA GAC GTT GCA    1122
Ala Asp Ala Leu Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala
                270                 275                 280

GAT AAA GGC TTA ACT TTA AAT ATT AAA TTC GCT GGT AAA AAA GAA AAA    1170
Asp Lys Gly Leu Thr Leu Asn Ile Lys Phe Ala Gly Lys Lys Glu Lys
            285                 290                 295

CCA GAA GAA CCA AAA GAA GAA GTT ACA ATC AAA GTT AAC TTA ATC TTT    1218
Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Val Asn Leu Ile Phe
        300                 305                 310

GCA GAT GGA AAG ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA    1266
Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu
    315                 320                 325

GCA ACA GCA AAA GCT TAT GCT TAT GCA GAC TTA TTA GCA AAA GAA AAT    1314
Ala Thr Ala Lys Ala Tyr Ala Tyr Ala Asp Leu Leu Ala Lys Glu Asn
330                 335                 340                 345
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FIG. 1 CONTINUED.

```
GGC GAA TAT ACA GCA GAC TTA GAA GAT GGT GGA AAC ACA ATC AAC ATT   1362
Gly Glu Tyr Thr Ala Asp Leu Glu Asp Gly Gly Asn Thr Ile Asn Ile
                350                 355                 360

                    C3
AAA TTT GCT GGA AAA GAA ACA CCA GAA ACA CCA GAA GAA CCA AAA GAA   1410
Lys Phe Ala Gly Lys Glu Thr Pro Glu Thr Pro Glu Glu Pro Lys Glu
            365                 370                 375

GAA GTT ACA ATC AAA GTT AAC TTA ATC TTT GCA GAT GGA AAG ATA CAA   1458
Glu Val Thr Ile Lys Val Asn Leu Ile Phe Ala Asp Gly Lys Ile Gln
        380                 385                 390

                    Z3
ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA AAA GCT TAT   1506
Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Lys Ala Tyr
    395                 400                 405

GCT TAT GCA AAC TTA TTA GCA AAA GAA AAT GGC GAA TAT ACA GCA GAC   1554
Ala Tyr Ala Asn Leu Leu Ala Lys Glu Asn Gly Glu Tyr Thr Ala Asp
410                 415                 420                 425

                                            C4
TTA GAA GAT GGT GGA AAC ACA ATC AAC ATT AAA TTT GCT GGA AAA GAA   1602
Leu Glu Asp Gly Gly Asn Thr Ile Asn Ile Lys Phe Ala Gly Lys Glu
                430                 435                 440

ACA CCA GAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACA ATC AAA GTT   1650
Thr Pro Glu Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Val
            445                 450                 455

AAC TTA ATC TTT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA   1698
Asn Leu Ile Phe Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly
        460                 465                 470

                Z4
ACA TTT GAA GAA GCA ACA GCA GAA GCT TAC AGA TAT GCA GAC TTA TTA   1746
Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu
    475                 480                 485

GCA AAA GTA AAT GGT GAA TAC ACA GCA GAC TTA GAA GAT GGC GGA TAC   1794
Ala Lys Val Asn Gly Glu Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr
490                 495                 500                 505

                                D1
ACT ATC AAC ATC AAA TTT GCT GGA AAA GAA CAA CCA GGC GAA AAT CCA   1842
Thr Ile Asn Ile Lys Phe Ala Gly Lys Glu Gln Pro Gly Glu Asn Pro
                510                 515                 520

GGA ATC ACA ATT GAT GAA TGG TTA TTA AAG AAT GCT AAA GAA GAA GCA   1890
Gly Ile Thr Ile Asp Glu Trp Leu Leu Lys Asn Ala Lys Glu Glu Ala
            525                 530                 535

ATC AAA GAA TTA AAA GAA GCA GGA ATC ACT TCT GAT TTA TAC TTC AGC   1938
Ile Lys Glu Leu Lys Glu Ala Gly Ile Thr Ser Asp Leu Tyr Phe Ser
        540                 545                 550
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FIG. 1 CONTINUED.

4/8

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TTA ATC AAT AAA GCA AAA ACA GTT GAA GGC GTA GAA GCA TTA AAG AAC  1986
Leu Ile Asn Lys Ala Lys Thr Val Glu Gly Val Glu Ala Leu Lys Asn
    555                 560                 565

GAA ATC TTA AAA GCA CAC GCT GGA GAA GAA ACA CCA GAA TTA AAA GAT  2034
Glu Ile Leu Lys Ala His Ala Gly Glu Glu Thr Pro Glu Leu Lys Asp
570                 575                 580                 585

GGA TAT GCA ACA TAT GAA GAA GCA GAA GCA GCA GCT AAA GAA GCT TTG  2082
Gly Tyr Ala Thr Tyr Glu Glu Ala Glu Ala Ala Ala Lys Glu Ala Leu
                590                 595                 600

AAA AAT GAT GAT GTT AAC AAC GCA TAC GAA ATA GTT CAA GGT GCA GAC  2130
Lys Asn Asp Asp Val Asn Asn Ala Tyr Glu Ile Val Gln Gly Ala Asp
            605                 610                 615

GGA AGA TAC TAC TAT GTA TTA AAG ATT GAA GTT GCA GAC GAA GAA GAA  2178
Gly Arg Tyr Tyr Tyr Val Leu Lys Ile Glu Val Ala Asp Glu Glu Glu
        620                 625                 630

CCA GGT GAA GAC ACT CCA GAA GTT CAA GAA GGT TAC GCA ACT TAC GAA  2226
Pro Gly Glu Asp Thr Pro Glu Val Gln Glu Gly Tyr Ala Thr Tyr Glu
    635                 640                 645

GAA GCA GAA GCA GCA GCT AAA GAA GCA TTA AAA GAA GAT AAA GTT AAC  2274
Glu Ala Glu Ala Ala Ala Lys Glu Ala Leu Lys Glu Asp Lys Val Asn
650                 655                 660                 665

AAT GCA TAC GAA GTA GTT CAA GGT GCA GAC GGA AGA TAC TAC TAT GTA  2322
Asn Ala Tyr Glu Val Val Gln Gly Ala Asp Gly Arg Tyr Tyr Tyr Val
                670                 675                 680

TTA AAA ATC GAA GAT AAA GAA GAT GAA CAA CCA GGT GAA GAA CCA GGC  2370
Leu Lys Ile Glu Asp Lys Glu Asp Glu Gln Pro Gly Glu Glu Pro Gly
            685                 690                 695

GAA AAC CCA GGA ATC ACA ATT GAT GAA TGG TTA TTA AAG AAT GCT AAA  2418
Glu Asn Pro Gly Ile Thr Ile Asp Glu Trp Leu Leu Lys Asn Ala Lys
        700                 705                 710

GAA GAC GCA ATC AAA GAA TTA AAA GAA GCA GGA ATC AGT TCT GAC ATA  2466
Glu Asp Ala Ile Lys Glu Leu Lys Glu Ala Gly Ile Ser Ser Asp Ile
    715                 720                 725

TAC TTT GAT GCA ATC AAC AAA GCA AAA ACA GTA GAA GGC GTA GAA GCG  2514
Tyr Phe Asp Ala Ile Asn Lys Ala Lys Thr Val Glu Gly Val Glu Ala
730                 735                 740                 745

TTA AAG AAC GAA ATC TTA AAA GCA CAC GCT GAA AAA CCA GGC GAA AAC  2562
Leu Lys Asn Glu Ile Leu Lys Ala His Ala Glu Lys Pro Gly Glu Asn
                750                 755                 760
```

E2

D2

D3

FIG. 1 CONTINUED.

```
CCA GGA ATC ACA ATT GAT GAA TGG TTA TTA AAG AAT GCT AAA GAA GCT    2610
Pro Gly Ile Thr Ile Asp Glu Trp Leu Leu Lys Asn Ala Lys Glu Ala
            765                 770                 775

GCA ATC AAA GAA TTA AAA GAA GCA GGA ATC ACT GCT GAA TAT CTA TTC    2658
Ala Ile Lys Glu Leu Lys Glu Ala Gly Ile Thr Ala Glu Tyr Leu Phe
        780                 785                 790

AAC TTA ATC AAC AAA GCA AAA ACA GTA GAA GGC GTA GAA TCA TTA AAG    2706
Asn Leu Ile Asn Lys Ala Lys Thr Val Glu Gly Val Glu Ser Leu Lys
    795                 800                 805

AAC GAA ATC TTA AAA GCA CAC GCT GAA AAA CCA GGC GAA AAC CCA GGA    2754
Asn Glu Ile Leu Lys Ala His Ala Glu Lys Pro Gly Glu Asn Pro Gly
810                 815                 820                 825

ATC ACA ATT GAT GAA TGG TTA TTA AAG AAC GCT AAA GAA GAT GCA ATT    2802
Ile Thr Ile Asp Glu Trp Leu Leu Lys Asn Ala Lys Glu Asp Ala Ile
                830                 835                 840

AAA GAA TTA AAA GAA GCA GGA ATT ACT TCT GAC ATA TAC TTT GAT GCT    2850
Lys Glu Leu Lys Glu Ala Gly Ile Thr Ser Asp Ile Tyr Phe Asp Ala
            845                 850                 855

ATC AAC AAA GCA AAA ACT ATT GAA GGC GTA GAA GCA TTA AAG AAT GAA    2898
Ile Asn Lys Ala Lys Thr Ile Glu Gly Val Glu Ala Leu Lys Asn Glu
        860                 865                 870

ATC TTA AAG GCT CAT AAA AAA GAT GAA GAA CCA GGT AAA AAA CCA GGT    2946
Ile Leu Lys Ala His Lys Lys Asp Glu Glu Pro Gly Lys Lys Pro Gly
    875                 880                 885

GAA GAC AAA AAA CCA GAA GAT AAA AAA CCA GGT GAA GAT AAA AAA CCA    2994
Glu Asp Lys Lys Pro Glu Asp Lys Lys Pro Gly Glu Asp Lys Lys Pro
890                 895                 900                 905

GAA GAC AAA AAA CCT GGT GAA GAT AAA AAA CCA GAA GAC AAA AAA CCA    3042
Glu Asp Lys Lys Pro Gly Glu Asp Lys Lys Pro Glu Asp Lys Lys Pro
                910                 915                 920

GGT AAA ACA GAT AAA GAT TCT CCA AAT AAG AAG AAA AAA GCT AAA TTA    3090
Gly Lys Thr Asp Lys Asp Ser Pro Asn Lys Lys Lys Lys Ala Lys Leu
            925                 930                 935

CCA AAA GCT GGT AGC GAA GCT GAA ATC TTA ACA TTA GCA GCA GCA GCT    3138
Pro Lys Ala Gly Ser Glu Ala Glu Ile Leu Thr Leu Ala Ala Ala Ala
        940                 945                 950

TTA TCA ACA GCA GCA GGT GCT TAC GTT TCA CTT AAA AAA CGT AAA TAATTAATCT
Leu Ser Thr Ala Ala Gly Ala Tyr Val Ser Leu Lys Lys Arg Lys        3193
    955                 960                 965

TAGATAAAGA ATAGATTAAT ATAAAAAATG GGACTTATAA TAGTCCCATT TTTTAATGCG  3253

AAAAACTGAT ACAAAAAATG TATCAG                                       3279
```

FIG. 1 CONTINUED.

C1
ATG GAA ACA CCA GAA CCA GAA GAA GAA GTT ACA ATC AAA GCT AAC TTA 48
Met Glu Thr Pro Glu Pro Glu Glu Glu Val Thr Ile Lys Ala Asn Leu
1 5 10 15

ATC TTT GCA GAT GGA AGC ACA CAA AAT GCA GAA TTC AAA GGA ACA TTC 96
Ile Phe Ala Asp Gly Ser Thr Gln Asn Ala Glu Phe Lys Gly Thr Phe
20 25 30

Z1
GCA AAA GCA GTA TCA GAT GCT TAC GCT TAC GCA GAT GCT TTA AAG AAA 144
Ala Lys Ala Val Ser Asp Ala Tyr Ala Tyr Ala Asp Ala Leu Lys Lys
35 40 45

GAC AAC GGA GAA TAT ACT GTA GAC GTT GCA GAT AAA GGC TTA ACT TTA 192
Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Leu Thr Leu
50 55 60

AAT ATT AAA TTC GCT GGT AAA C2 AAA GAA AAA CCA GAA GAA CCA AAA GAA 240
Asn Ile Lys Phe Ala Gly Lys Lys Glu Lys Pro Glu Glu Pro Lys Glu
65 70 75 80

GAA GTT ACA ATC AAA GTT AAC TTA ATC TTT GCA GAT GGA AAG ACA CAA 288
Glu Val Thr Ile Lys Val Asn Leu Ile Phe Ala Asp Gly Lys Thr Gln
85 90 95

ACA GCA GAA TTC AAA GGA ACA TTT Z2 GAA GAA GCA ACA GCA AAA GCT TAT 336
Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Lys Ala Tyr
100 105 110

GCT TAT GCA GAC TTA TTA GCA AAA GAA AAT GGC GAA TAT ACA GCA GAC 384
Ala Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Glu Tyr Thr Ala Asp
115 120 125

TTA GAA GAT GGT GGA AAC ACA ATC AAC ATT AAA TTT GCT GGA C3 AAA GAA 432
Leu Glu Asp Gly Gly Asn Thr Ile Asn Ile Lys Phe Ala Gly Lys Glu
130 135 140

ACA CCA GAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACA ATC AAA GTT 480
Thr Pro Glu Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Val
145 150 155 160

FIG.2

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                                                    7/8
AAC TTA ATC TTT GCA GAT GGA AAG ATA CAA ACA GCA GAA TTC AAA GGA      528
Asn Leu Ile Phe Ala Asp Gly Lys Ile Gln Thr Ala Glu Phe Lys Gly
                  165                 170                 175

                  Z3
ACA TTT GAA GAA GCA ACA GCA AAA GCT TAT GCT TAT GCA AAC TTA TTA      576
Thr Phe Glu Glu Ala Thr Ala Lys Ala Tyr Ala Tyr Ala Asn Leu Leu
          180                 185                 190

GCA AAA GAA AAT GGC GAA TAT ACA GCA GAC TTA GAA GAT GGT GGA AAC      624
Ala Lys Glu Asn Gly Glu Tyr Thr Ala Asp Leu Glu Asp Gly Gly Asn
        195                 200                 205

                        C4
ACA ATC AAC ATT AAA TTT GCT GGA AAA GAA ACA CCA GAA ACA CCA GAA      672
Thr Ile Asn Ile Lys Phe Ala Gly Lys Glu Thr Pro Glu Thr Pro Glu
    210                 215                 220

GAA CCA AAA GAA GAA GTT ACA ATC AAA GTT AAC TTA ATC TTT GCA GAT      720
Glu Pro Lys Glu Glu Val Thr Ile Lys Val Asn Leu Ile Phe Ala Asp
225                 230                 235                 240

                                                Z4
GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA      768
Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr
                245                 250                 255

GCA GAA GCT TAC AGA TAT GCA GAC TTA TTA GCA AAA GTA AAT GGT GAA      816
Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Val Asn Gly Glu
          260                 265                 270

TAC ACA GCA GAC TTA GAA GAT GGC GGA TAC ACT ATC AAC ATC AAA TTT      864
Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr Thr Ile Asn Ile Lys Phe
        275                 280                 285

GCT GGA AAA TAA                                                     876
Ala Gly Lys  *
    290
```

FIG. 2 CONTINUED

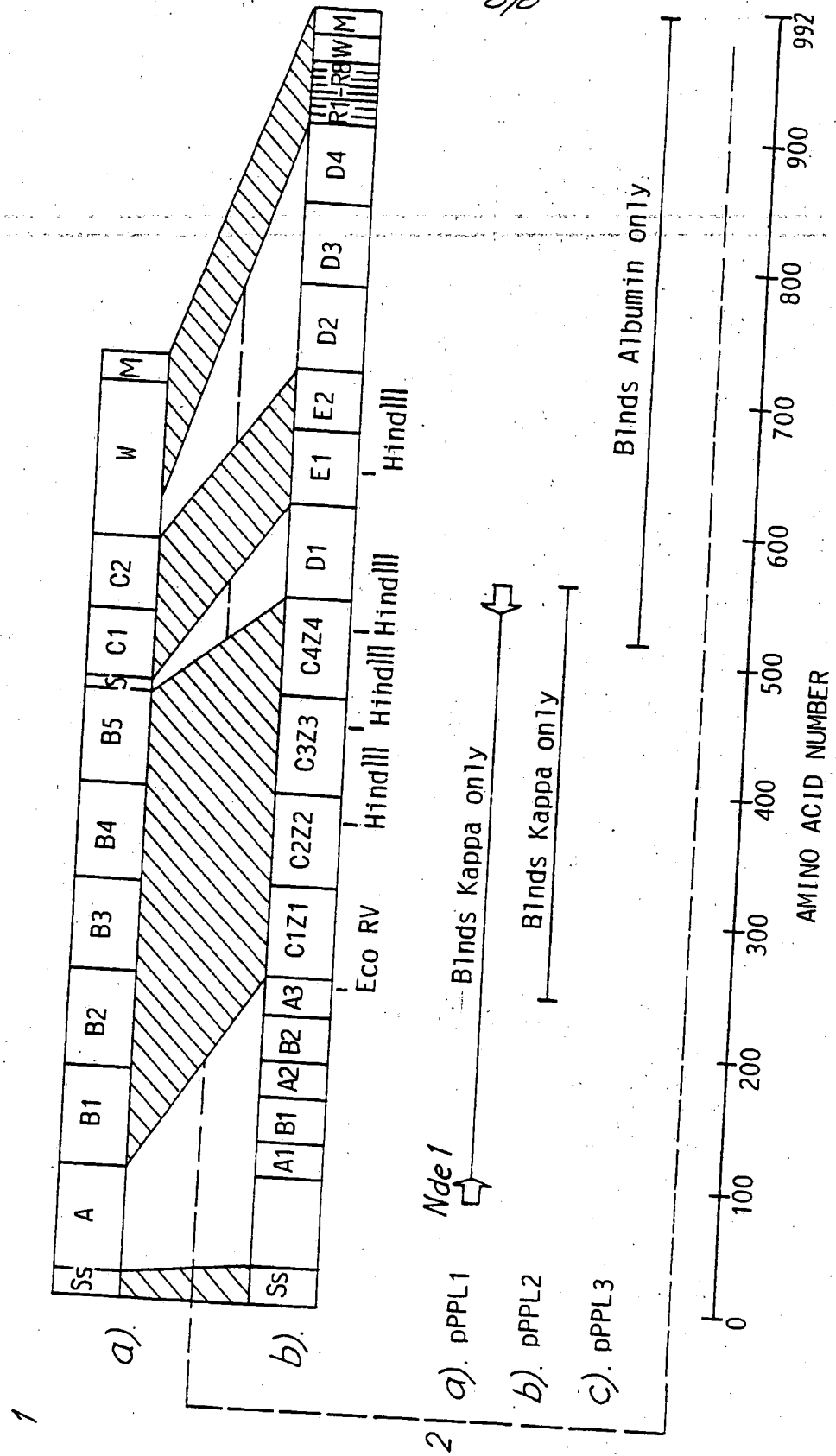
a).
b).
Ss
A
B1
B2
B3
B4
B5
C1
C2
W
M
A1
A2
A3
C1Z1
C2Z2
C3Z3
C4Z4
D1
E1
E2
D2
D3
D4
R1
R2
W
M
Eco RV
HindIII
HindIII
HindIII
HindIII
Nde 1
1
2
a). pPPL1
b). pPPL2
c). pPPL3
Blnds Kappa only
Blnds Kappa only
Blnds Albumin only
0
100
200
300
400
500
600
700
800
900
992
AMINO ACID NUMBER

FIG.3

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00950

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/31; C07K13/00; C12N15/62

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 267, no. 4, 5 February 1992, BALTIMORE, MD US pages 2234 - 2239 Nilson BH;Solomon A;Bjorck L;Akerstrom B; 'Protein L from Peptostreptococcus magnus binds to the kappa light chain variable domain.' see the whole document ---	1-16
A	INFECTION AND IMMUNITY vol. 58, no. 5, May 1990, WASHINGTON US pages 1217 - 1222 Kastern W;Holst E;Nielsen E;Sjobring U;Bjorck L; 'Protein L, a bacterial immunoglobulin-binding protein and possible virulence determinant.' see the whole document --- -/--	1-16

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 JULY 1993	29 -08- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

Form PCT/ISA/210 (second sheet) (January 1985)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 33, 25 November 1989, BALTIMORE, MD US pages 19740 - 19746 Akerstrom B;Bjorck L; 'Protein L: an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties.' see the whole document	1-16
A	EP,A,0 255 497 (HIGHTECH RECEPTOR AB, S) 3 February 1988 cited in the application see the whole document	1-16

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300950
SA 73685

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 28/07/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0255497	03-02-88	US-A- 4876194	24-10-89
		JP-A- 63032372	12-02-88

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82